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PRINCIPAL INVESTIGATOR: F. Carter Bancroft, Ph.D.

CONTRACTING ORGANIZATION: Mount Sinai Medical Center
New York, New York 10029-6574

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F. Carter Bancroft, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Mount Sinai Medical Center
New York, New York 10029-6574

E-mail: cbancro@smtplink.mssm.edu

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The purpose of the research is to clone, isolate and characterize protein markers that are specifically expressed by advanced stage, androgen-independent prostate cancers. Two types of markers with this specificity are expected to be identified: (1) proteins secreted specifically by advanced stage prostate cancer cells, potentially useful in serum-based assays designed to specifically detect this class of tumors; and (2) markers expressed specifically on the surface of these cells , potentially useful as specific targets for clinical treatment of androgen-independent prostate cancer. Progress to date has involved: (i) preparation of membrane fraction mRNA's from four human cell lines representing four stages of prostate cancer, and use of these mRNA's to prepare cDNA libraries; (ii) isolation of proteins from membrane fractions of two of these cell lines, and initiation of a collaboration to employ the techniques of Proteomics to differentially analyze these protein populations. We can now apply the new DNA microarray technology to the cDNA libraries described in (i) to identify clones possessing the characteristics described above. In addition, we are now employing the proteins described in (ii) above for differential screening via two-dimensional gel analysis, to directly identify cell surface proteins specifically expressed by advanced stage prostate cancer cells.

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FOREWORD

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_NA_ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Carter Bancroft 8/26/99

PI - Signature Date

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(5) INTRODUCTION

This project is directed to the problem of androgen-independent prostate cancer, which is a major cause of cancer-related mortality among men. The purpose of the research is to clone, isolate and characterize protein markers that are specifically expressed by advanced stage, androgen-independent prostate cancer. Two types of markers with this specificity are expected to be identified: (1) proteins secreted specifically by advanced stage prostate cancer cells, which may prove useful in serum-based assays designed to detect this particular class of tumors; and (2) markers expressed specifically on the surface of advanced stage prostate cancer cells, which may prove useful as targets for clinical treatment of androgen-independent prostate cancer.

(6) BODY

As indicated by the title of the grant application, the central aim of this project is to isolate and characterize prostate cancer cell markers. As described in more detail in the application (Statement of Work, Task 1), we are employing an approach that will narrow down the protein markers identified to those that are: 1. specifically expressed by androgen-independent prostate cancer cells; and 2. are likely to represent clinically significant markers by virtue of being either secreted by cells of this type, or expressed on the surface of these cells. In the work during this first year of the grant, we have concentrated upon Task 1 of the Statement of Work.

We had originally proposed to employ only two related human prostate cancer cell lines in our studies, corresponding to two stages of prostate cancer: the LNCaP

androgen-dependent cells, and one of its androgen-independent sublines, termed C4-2 cells. However, we have actually been able to obtain and work with four cell lines in this lineage, corresponding to four major stages in prostate cancer progression: the androgen- and bone-dependent **LnCaP** cells, their bone-independent **C4** cell derivatives, the C4-derivative bone- and androgen-independent **C4-2** cells, and the C4-2-derivative **C4-2b** cells, which exhibit bone metastasis. Although our work with these four cell lines was initially somewhat delayed by difficulties encountered in propagation of these difficult cell lines, we have now successfully grown all four of them, and employed them as described below. In addition, at the time of the grant application, we had intended to use for our studies what was then the most advanced technology for differential gene isolation: subtractive hybridization. However, in the almost two years since submission of the application for this grant, two new technologies have appeared and now represent cutting edge technologies for carrying out differential gene isolation. We are embracing each of these technologies, termed respectively DNA Microarray Technology and Proteomics, in our work under this grant. Our work to date toward the use of each of these technologies to address the research topic of this grant is described separately below:

DNA Microarray Technology. This novel and powerful technology involves the use of arrayed libraries of cDNA clones to carry out high throughput screening of gene expression in normal and pathological tissue. DNA microarray technology promises to displace all previous techniques designed to employ differential gene expression to isolate genes exhibiting disease-specific expression. The PI of this grant, Dr. Bancroft,

is presently spearheading the effort to establish this technology at The Mount Sinai School of Medicine.

In preparation for the availability of this technology here, we have, as described in Task 1, isolated from C4-2 and LNCaP cells, mRNA populations enriched for mRNA's encoding either cell surface markers or secretory proteins, and used these mRNA's to prepare cDNA libraries. As described above, we have extended this Task by also preparing comparable cDNA libraries from the C4 and C4-2b cell lines. We now plan to prepare arrayed libraries of cDNA clones from each of these four cell lines, and then employ probes prepared from the cell lines corresponding to the most advanced stages of prostate cancer (the C4-2 and C4-2b cells) to identify and isolate genes expressed specifically in advanced stage prostate cancer. These will then be further characterized as described in the Statement of Work.

Proteomics. Our work with this technology represents a novel, additional way to address the original research topic. Proteomics involves analysis of gene expression via studies of the protein products of the genes. Analysis is usually performed by two-dimensional gel electrophoresis. As we pointed out in the original application, the membrane fraction of prostate cancer cells should be enriched for mRNA's encoding either secreted proteins or cell surface proteins. We have since realized that this same membrane fraction should represent an excellent source of the cell surface proteins themselves. Thus, by carrying out comparative two-dimensional gel analysis of membrane fraction proteins from the cell lines described above, it should be possible to identify markers displayed specifically on the surface of advanced stage prostate cancer

cells. As described in the original application, such proteins represent potential therapeutic targets.

We have now prepared membrane fractions all four of the cell lines described above, and have isolated proteins from these fractions. We are now engaged in a collaboration with Dr. Marc Glucksman of the Neurobiology Center at Mount Sinai, who is an expert on Proteomics and two-dimensional gel analysis, to analyze these proteins to identify surface markers expressed specifically by the C4-2B cells. In addition, we have just begun planning with Ciphergen Biosystems, Inc. to carry out collaborative protein differential display studies of these protein samples, employing the SELDI ProteinChip technology that Ciphergen has developed. Proteins found to be expressed specifically by the C4-2B cells, corresponding to advanced stage prostate cancer, will then be further characterized as described in the Statement of Work. However, in this case, we will have in hand the proteins, and will thus need to work backward to isolate the corresponding genes from an appropriate cDNA library.

(7) KEY RESEARCH ACCOMPLISHMENTS

- Isolated, from LNCaP, C4, C4-2, and C4-2b human prostate cancer cell lines, mRNA populations enriched for either cell surface markers or secretory proteins.
- Used the mRNA isolated from each cell line to prepare a cDNA library.
- Prepared membrane fractions from the LNCaP and C4-2B cells, and isolated proteins from these fractions.

- Initiated collaborative investigations to employ comparative two-dimensional gel analysis of these proteins to search for cell surface marker proteins expressed specifically by the C4-2B cells.

(8) REPORTABLE OUTCOMES

None.

(9) CONCLUSIONS

During the past year, we have carried out experiments that have set the stage for using two recently developed, powerful technologies in pursuit of the central aim of this project: to clone, isolate, and characterize clinically significant prostate cancer markers. We will now apply DNA microarray technology to the specialized cDNA libraries we have prepared from four human prostate cancer cells, corresponding to four stages of prostate cancer. This work will involve differential screening of microarrays prepared from each cDNA library, to identify cDNA's that represent genes possessing the following dual properties: the genes are expressed specifically in advanced stage prostate cancer, and their gene products are either secretory proteins or cell surface marker proteins. At the same time, we will continue to apply the techniques of Proteomics to the membrane proteins we have isolated from human prostate cancer cell lines, to identify and isolate cell surface proteins expressed specifically by late stage prostate cancer cells.

In our work during the first year of the project, we have not concentrated upon Task 2, for the following reasons. Dr. Brian Liu, who was expected to be largely

responsible for this task, has left the Mount Sinai School of Medicine. We have not been successful in finding a suitable replacement for Dr. Liu. In addition, the direct Government Cost Share of the annual budget was cut from the originally proposed amount of $124,876 to $100,295, making it very difficult to simultaneously pursue Tasks 1 and 2. Finally, and probably most significantly, as described above, since submission of the application for this grant, two novel technologies have become available for pursuing the central purpose of this project: to clone and isolate novel prostate cancer cell markers. We thus propose in the coming year to continue to employ the power of these technologies to concentrate upon Task 1 and then its follow-up Task 3 in preference to Task 2 and its dependent Task 4. We believe that, with the availability of these powerful technologies, Tasks 1 and 3 together now represent the most direct route to achievement of the Statement of Work of this project.